

Ion PGM™ Checklist — Ion OneTouch™ 2 Template Preparation

Pub. no. MAN0009128

Rev. 1.0

Use this checklist if you are an experienced user of the Ion PGM™ Template OT2 200 Kit (Catalog no. 4480974).

For complete kit and protocol information, including precautions and safety information, refer to the *Ion PGM™ Template OT2 200 Kit User Guide* (Pub no. MAN0007220), available on the [Ion Community](#) website.

Set up the Ion OneTouch™ 2 Instrument

Check the firmware

- Check the firmware. Update to the latest firmware version if a later version is available.

Install the Ion OneTouch™ Recovery Tubes and Ion OneTouch™ Recovery Router

- Install the 2 Recovery Tubes and the Recovery Router, then close the centrifuge lid.

Install the Ion OneTouch™ 2 Amplification Plate



1. If there is a used cleaning adapter on the instrument, remove and appropriately discard it. Open the heat block.
2. Hold the disposable injector in one hand and the Amplification Plate in the other hand.
3. Insert the Amplification Plate into the heat block, then close the heat block and thread and install the disposable tubing.

Install the disposable injector

1. Insert the disposable injector until the injector stops at the router base. Release the disposable injector.
2. Confirm automatic placement of the disposable injector by pressing then releasing the spring-loaded top of the Injector Hub.

Note: You should hear a click and the Injector Hub should return to the up position.

Install the reagents

1. If you are using a new kit, appropriately discard of the Recovery Solution and used Reagent Tubes and contents.
2. Use fresh gloves to install new sipper tubes.
3. Install Ion OneTouch™ Oil on the left front port : invert the bottle 3 times, fill the Reagent Tube *half*-full, and install the tube.
4. Install Recovery Solution on the right front port : invert the bottle 3 times, fill the Reagent Tube *quarter*-full, and install the tube.

Empty the waste container and the oil waste tray

1. Empty the waste container and reattach the tubing to the container.
2. Slowly pull out, but do not remove, the oil waste tray. If there is no oil, reinsert the tray. Dispose of any excessive oil.

Prepare the amplification solution (in a pre-PCR dedicated room or hood)

1. Prepare the Reagent Mix, PCR Reagent B, Enzyme Mix, and library.
2. In a 1.5-mL Eppendorf LoBind® Tube, add the following components in order and pipet up and down after adding each component:

Order	Reagent	Cap color	Volume
<input type="checkbox"/> 1	Nuclease-Free Water	—	25 µL
<input type="checkbox"/> 2	Ion PGM™ Template OT2 200 Reagent Mix	Violet	500 µL
<input type="checkbox"/> 3	Ion PGM™ Template OT2 200 PCR Reagent B	Blue	300 µL
<input type="checkbox"/> 4	Ion PGM™ Template OT2 200 Enzyme Mix	Brown	50 µL
<input type="checkbox"/> 5	Freshly prepared, diluted library (<i>not</i> stock library)	—	25 µL
—	Total	—	900 µL

3. Vortex the solution at maximum speed for 5 seconds, then centrifuge for 2 seconds.
4. Prepare the Ion Sphere™ Particles (ISPs) by vortexing for 1 minute, briefly centrifuging, and pipetting to mix.
5. Add 100 µL of ISPs to the 900 µL amplification solution, and vortex for 5 seconds.

Fill and install the Ion PGM™ OneTouch Plus Reaction Filter Assembly on the Ion OneTouch™ 2 Instrument

1. Place a Plus Reaction Filter Assembly into a tube rack so that the 3 ports of the filter face up.
2. Vortex the amplification solution at maximum speed for a *full 5 seconds* and centrifuge the solution for 2 seconds.
3. Slowly pipet the entire 1000 µL of solution through the sample port.
4. Slowly pipet 1000 µL of Ion OneTouch™ Reaction Oil into the sample port; change tip, then slowly add another 500 µL of oil.
5. Invert Reaction Filter Assembly: Rotate the assembly to your *right* until the reaction tube is inverted and the 3 ports face *down*.
6. Firmly insert the 3 ports into the three holes on the top of the Ion OneTouch™ 2 Instrument.

Run the Ion OneTouch™ 2 Instrument

1. Ensure that the centrifuge lid of the Ion OneTouch™ 2 Instrument is closed. Touch **Run**.
2. Touch the drop-down menu, then select **PGM: Ion PGM™ Template OT2 200 Kit**. Touch **Next**.
3. Choose **Assisted** or **Expert**. Complete the listed tasks, then touch **Next**.

Recover the Ion PGM™ Template OT2 200 Ion Sphere™ Particles

1. At the end of the run, follow prompts to centrifuge the sample if more than 15 minutes have elapsed since the end of the run.
2. After the centrifuge stops, touch **Open Lid**, wait until the lid opens, then remove and discard the Recovery Router.
3. Carefully remove both Recovery Tubes, and put the tubes in a rack.
4. Remove and discard all but 50 µL of the Recovery Solution from each Recovery Tube without disturbing the pellet.
5. With a new tip, resuspend the ISPs in the remaining Recovery Solution by pipetting the pellet up and down.
6. Assess the quality of a 2 µL aliquot of the unenriched, template-positive ISPs using a Qubit® 2.0 Fluorometer or Flow Cytometer.
7. Enrich template-positive ISPs: proceed to the *Ion PGM™ Checklist — Ion One Touch™ ES Enrichment (MAN0009379)* or store ISPs.
Note: To store the ISPs: combine suspensions, add 1 mL of Ion OneTouch Wash Solution, and store at 2–8°C.

Maintain the Ion OneTouch™ 2 Instrument

1. Ensure that there is ≥20 mL of Ion OneTouch™ Oil (in *left* Reagent Tube). If not, add oil into the Reagent Tube until it is half-full.
2. Remove and discard the used Reaction Filter Assembly.
3. Keep the Ion OneTouch™ 2 Amplification Plate in the heat block.
4. Firmly insert the 3 ports of the single-use Cleaning Adapter into the 3 holes on the top of the instrument.
5. Remove the disposable injector from the Injector Hub and remove the disposable tubing from the pinch valve.
6. Place the used injector into an empty 50-mL conical tube in a tube rack by the instrument.
7. On the instrument home screen, touch **Clean**. Complete each task, then touch **Next**.
8. When cleaning is complete, dispose of the waste in the 50-mL conical tube.
9. Remove and dispose of the used Amplification Plate, disposable injector, and tubing from the instrument.
10. Touch **Open Lid** to open the centrifuge lid. Wipe the residue from the centrifuge lid with a disposable wipe, then close the lid.
11. Touch **Next** to return to the home screen on the instrument.

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